

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
United States Patent and Trademark  
Office  
Box PCT  
Washington, D.C.20231  
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

<b>Date of mailing</b> (day/month/year) 04 October 2000 (04.10.00)	<b>Applicant's or agent's file reference</b> 49435	<b>RECEIVED</b> JUL 20 2001
<b>International application No.</b> PCT/FI00/00031	<b>Priority date</b> (day/month/year) 18 January 1999 (18.01.99)	
<b>International filing date</b> (day/month/year) 18 January 2000 (18.01.00)	<b>Applicant</b> SEPPÄNEN, Helena et al	

TECH CENTER 1600-2900

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

18 August 2000 (18.08.00)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO  
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## PATENT COOPERATION TREATY

REC'D 08 MAY 2001

## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 49435	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/FI00/00031	International filing date (day/month/year) 18/01/2000	Priority date (day/month/year) 18/01/1999
International Patent Classification (IPC) or national classification and IPC G01N33/543		<b>RECEIVED</b> JUL 20 2001 TECH CENTER 1600/2900
Applicant [LABSYSTEMS OY et al.] THERMO LABSYSTEMS OY et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 6 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 18/08/2000	Date of completion of this report 04.05.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Moreno de Vega, C Telephone No. +49 89 2399 7486 

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/FI00/00031

## I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, pages:**

1-8 as originally filed

**Claims, No.:**

1-14 as originally filed

**Drawings, sheets:**

1/4-4/4 as originally filed

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2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/FI00/00031

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

## IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.  
☐ paid additional fees.  
☐ paid additional fees under protest.  
☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.  
☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.  
☐ the parts relating to claims Nos. .

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 3-6
	No:	Claims 1,2,7-14
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-14

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/FI00/00031

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Industrial applicability (IA)    Yes:    Claims    1-14  
   No:    Claims

2. Citations and explanations  
    **see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:  
**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/FI00/00031

**Re Item IV**

**Lack of unity of invention**

This Examining Authority agrees with the objection of lack of unity raised in the International Search Report for the reasons given therein.

**Re Item V**

**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

D1: WO-A2-9618731

D2: US-A-5705628

D3: WO 98/31840

**1. Novelty (Article 33(2) PCT)**

The applicant's observations in reply to the Written opinion have been considered, and the differences of the present invention with the prior art methods have been recognised. However, the following objections are maintained:

D1 (see page 6 line 23 - page 12 line 9, Example 1) discloses a method of isolating nucleic acids from a sample, said method comprising contacting said sample with a detergent and a solid support, i.e. magnetic beads, and separating said support with bound nucleic acid from the sample.

D2 (see column 1 line 24 - column 2 line 60, column 5 line 29 - column 6 line 27) describes a method of separating polynucleotides from a solution by binding the polynucleotides to a solid surface, such as magnetic microparticles. The magnetic microparticles with bound DNA are washed with a suitable elution buffer, containing, i.e. salt, sucrose or formamide, and separated from the solution applying a magnetic field or by filtration.

D3 (see page 7 lines 9-21, page 8 lines 2-7, page 8 line 30-page 9 line 12, page 12 line 1 - page 13 line 8, page 17 line 20-page 21 line 12, claims)

discloses methods for isolating biological target materials from other substances in a medium using silica magnetic particles, forming a complex of the silica magnetic particles and the biological target material in a mixture of the medium and particles, separating the complex from the mixture using external magnetic force, and eluting the biological target material from the complex. A magnetizable probe is inserted into the solution, such that the particles deposit on the end of the probe immersed in the solution, the probe is removed from the solution and immersed into a second solution. To the medium containing the biological material a chaotropic salt is added. This document appears to be novelty destroying for claims 1,2,7-14.

Thus, claims 1,2, 7-14 do not meet the requirements of Article 33(2) PCT.

**2. Inventive step (Article 33(3) PCT)**

Claims 3-6 differ from D3, which is considered to be the most relevant prior art in that proteins and detergents are used as surface tension releasing compounds in a method for purification of a substance using magnetic particles which bind to the substance. The technical problem to be solved by these claims is the provision of an improved method for the purification of a substance in a medium. The solution provided by the present invention is based on the addition of surface tension releasing agent to the medium and the use of magnetic probes which are pushed into the medium whereby the particles adhere to the probes and are separated from the medium. This solution is the same proposed in D3 to solve the same technical problem. In the light of D1 and D2 the person skilled in the art would have used detergents and other substances with known properties as surface tension releasing agents like casein without exercising inventive skill, arriving at the method of claims 3-6.

Thus, claims 3-6 do not meet the requirements of Article 33(3) PCT.

**Re Item VII**

**Certain defects in the international application**

It seems that in claims 9 and 10, instead of "purification" it should read "purification", and in claim 14 instead of "adhered" it should read "adhered".

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>G01N 33/543</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/42432</b> <b>(43) International Publication Date:</b> 20 July 2000 (20.07.00)
<b>(21) International Application Number:</b> PCT/FI00/00031 <b>(22) International Filing Date:</b> 18 January 2000 (18.01.00)  <b>(30) Priority Data:</b> 990082 18 January 1999 (18.01.99) FI  <b>(71) Applicant (for all designated States except US):</b> LABSYS- TEMS OY [FI/FI]; P.O. Box 8, FIN-00881 Helsinki (FI).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SEPPÄNEN, Helena [FI/FI]; Linnanherrantie 7 A 6, FIN-00950 Helsinki (FI). PALOMÄKI, Pekka [FI/FI]; Alakartanontie 7 B 17, FIN-02360 Espoo (FI). TUUNANEN, Jukka [FI/FI]; Koivikkotie 20 C, FIN-00630 Helsinki (FI). KÄRMENIEMI, Timo [FI/FI]; Punahilkantie 5 A 7, FIN-00820 Helsinki (FI).  <b>(74) Agent:</b> BERGGREN OY AB; P.O. Box 16, FIN-00101 Helsinki (FI).		<b>(81) Designated States:</b> JP, NO, RU, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PURIFICATION PROCESS USING MAGNETIC PARTICLES		
<b>(57) Abstract</b>  The invention relates to a process for the purification of a substance with magnetic particles treated with a reagent which binds the particles. After a binding reaction in a first medium, the particles and the substance bound to them are separated and transferred to a second medium. According to the invention, a surface tension releasing agent is dispensed at least the mediums before the particles are separated from it. This promotes the complete collection of the particles.		



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## PURIFICATION PROCESS USING MAGNETIC PARTICLES

### Background of the invention

5 The present invention relates to the purification of biological substances using magnetic particles which bind the material specifically from a mixture. The invention can be used for instance for purifying nucleic acids DNA or RNA.

10 Magnetic particles can be coated with a separation reagent which reacts specifically with a desired biological substance. The particles and the bound substance are separated from the mixture and thereafter the substance is released from the particles for further prosecution. Nowadays this is done in practice so that the particles are drawn with a magnet against the wall of the vessel containing the mixture, and the liquid is poured or sucked off the vessel. Thereafter a new liquid can be dispensed into the vessel. Manual or automatic equipments for such separation technology are also commercially available (e.g., Spherotec, Inc., 15 AutoMag Processor (USA), Merck Magnetic Rack (Darmstadt, Germany), PerSeptive Biosystems 96 well plate separator, Multi-6 Separator, Solo-Sep Separator (USA), Dynal Magnetic Particles Concentrators).

20 The old purification technique for DNA involves ultracentrifugation in a dense cesium chloride gradient. However, also magnetic particle technology described above has been used for purifying nucleic acids.

25 WO 94/18565 (Labsystems Oy) suggests a method and device for magnetic particle specific binding assay, in which magnetic particles are separated from a mixture by a probe comprising a rod movable in a vertical bore and provided with a magnet at the lower end thereof. The probe is pushed into the mixture with the rod in the lower position, whereby the particles are collected on the probe. Then the probe is transferred to another vessel and the rod is pulled in its upper position, whereby the particles are released. Thus all steps of the assay can be carried out in a separate vessel without having to transfer liquids. In the last vessel, a measurement is carried out.

30 WO 96/12959 (Labsystems Oy) suggests a magnetic particle transfer tool comprising an elongated body with a concavely tapered tip part. The body further comprises means for providing a longitudinal magnetic field to collect particles to the tip of the body. The magnetic field can be eliminated in order to release the

particles. This tool can be used especially for collecting particles from a large volume and releasing them into a very small volume.

### General description of the invention

5 Now a method according to claim 1 has been invented. Some preferable embodiments of the invention are defined in the other claims.

10 According to the invention, material to be purified is dispensed in a first medium containing magnetic particles, which have been coated with a binding reagent for the material. The binding reaction takes place, after which the particles are separated by means of a magnetic probe and transferred into a second medium, in which a desired further reaction necessary for the purification may take place. The particles can be transferred similarly via further mediums for carrying out further steps of the purification process. All the vessels may contain the necessary reaction medium ready when the particles are transferred into it. Preferably the particles are also released from the probe in the second and subsequent mediums.

15 According to the invention, at least one of the mediums contains a surface tension releasing agent. This promotes the complete collection of the particles.

20 Some of such agents have been used also before in this connection to promote the binding of the substance to be separated, see e.g. Wipat et al., Microbiology (1994), 140, 2067. In these known methods, the particles are not transferred from a vessel to another but they are held on the wall of the vessel by means of an external magnet, while the medium is removed from the vessel.

The invention can be used especially for purifying nucleic acids, such as ssDNA, dsDNA, and mRNA.

### Brief description of the drawings

25 The enclosed drawings form a part of the written description.

Figure 1 shows the effect of a detergent in collecting and releasing steps of magnetizable particles.

Figure 2 shows the effect of salt and saccharose in collecting and releasing buffer.

Figure 3 shows the effect of protein in collecting and releasing buffer.

30 Figure 4 shows the effect of a detergent when magnetic particles of different suppliers were used.

### Detailed description of the invention

The invention can be used for instance for the purification of cells, viruses, subcellular organelles, proteins, and especially nucleic acid materials.

5 The magnetic particles are preferably paramagnetic. The size of the particles is usually less than 50  $\mu\text{m}$ , preferably 0.1 - 10  $\mu\text{m}$ , and most preferably 1 - 5  $\mu\text{m}$ . The concentration of the particles may be eg. 0.01 - 5 mg/ml, preferably 0.05 - 3 mg/ml, and most preferably 0.2 - 2 mg/ml.

10 The particles have been coated or treated with a binding reagent, eg. silicon, lectins and/or other reactive functional groups such as oligonucleotides, antibodies, antigens, streptavidin, or biotin.

15 The particles are preferably transferred from a vessel to another by using a probe comprising a rod movable in a vertical bore and provided with a magnet at the lower end thereof. The probe is pushed into the mixture with the rod in the lower position, whereby the particles are collected on the probe. Then the probe is transferred to another vessel, and when the rod is pulled to its upper position the particles are released.

Different kind of surface tension releasing compounds, especially water soluble compounds, can be used in the method. Examples of such are:

A. Tensides, such as

- 20 - Soaps  
- Detergents; including anionic, kationic, non-ionic and zwitterionic compounds

B. Alcohols, such as

- Polyethylene and polyvinyl alcohols and their protein etc. derivatives

C. Proteins

25 D. Salts and carbohydrates in high concentrations, such as

- NaCl  
- Saccharose

Also mixtures of compounds can be used.

30 Especially tensides such as detergents are suitable. Preferable detergents are ethoxylated anhydrosorbitol esters. The esters may contain eg. about 4 - 20 ethylene oxide groups.

The concentration of a tenside may be eg. 0.001 - 0.5% (w/v), preferably 0.005 - 0.1 % (w/v), and most preferably 0.01 - 0.05% (w/v). The concentration of a protein

may be eg. 0.1 - 10% (w/v), preferably 0.25 - 5% (w/v), and most preferably 0.5 - 2% (w/v). The concentraion of a salt may be eg. 0.1 - 10 M, preferably 0.1 - 7 M.

For purification of DNA or mRNA from different sources (for instance, DNA from PCR amplification; DNA from blood, bone marrow or cultured cells; mRNA from eucaryotic total RNA or from crude extracts of animal tissues, cells and plants) the nucleic acids are immobilized by using magnetic particles. The binding can be mediated by the interaction of streptavidin and biotin, whereby particles coated with streptavidin and biotinylated DNA can be used. In addition, DNA can be adsorbed to the surface of the particles. The binding of mRNA can be mediated by Oligo (dT)<sub>25</sub> covalently coupled to the surface of the particles.

After the immobilization, the nucleic acids are washed several times to remove all the reaction components resulting from the amplification or other contaminants and, e.g., PCR inhibitors.

The washing can be performed by releasing and collecting complexes in a washing buffer and by transferring the complexes to another well containing fresh washing buffer.

For ssDNA purification the immobilized double-stranded DNA can be converted to a single-stranded by incubation with 0.1 M NaOH and using magnetic separation.

For the isolation of mRNA, it can be eluted from the particles by using a low salt buffer.

The purification process can be performed by a magnetic particle processor, in which all the mediums are ready in separate vessel. A surface tension releasing compound is preferably used in each medium. Suitable disposable plates, such as microtitration plates, comprising the necessary vessels can be used. In one plate, several parallel purifications can be accomplished.

#### **Example of reagents used for a ssDNA purification**

1. Particle suspension in eg. phosphate, Tris or Borate buffered saline, pH 7.4, containing 0.1% BSA, 15 mM NaN<sub>3</sub> and eg. 0.02% polyoxyethylene (20) sorbitan monolaurate (Tween 20<sup>TM</sup>) as a surface tension releasing agent
2. Binding and Washing buffer (TEN): 10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl, eg. 0.02% Tween 20<sup>TM</sup>, 15 mM NaN<sub>3</sub>, pH 7.5

3. TE buffer: 10 mM Tris-HCl, 1 mM EDTA, eg. 0.02% Tween 20<sup>TM</sup>, 15 mM NaN<sub>3</sub>, pH 7.5

4. Melting solution: 0.1 M NaOH, eg. 0.02% Tween 20<sup>TM</sup>

5. eg. 0.02% Tween 20<sup>TM</sup> in distilled water, 15 mM NaN<sub>3</sub>

#### 5 Example of reagents used for a mRNA direct purification

1. Oligo (dT)<sub>25</sub> particle suspension in PBS, pH 7.4, containing eg. 0.02% Tween 20<sup>TM</sup> and 15 mM NaN<sub>3</sub>

2. Lysis/binding buffer: 100 mM Tris-HCl, pH 8.0, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM dithiothreitol (DTT), 15 mM NaN<sub>3</sub>, (eg. 0.02% Tween 20<sup>TM</sup>)

10 3. Washing buffer with LiDS (SDS): 10 mM Tris-HCl, pH 8.0, 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS, 15 mM NaN<sub>3</sub> (eg. 0.02% Tween 20<sup>TM</sup>)

4. Washing buffer: 10 mM Tris-HCl, pH 8.0, 0.15 M LiCl, 1 mM EDTA, eg. 0.02% Tween 20<sup>TM</sup>, 15 mM NaN<sub>3</sub>

5. Elution solution: 2 mM EDTA, pH 8.0, 15 mM NaN<sub>3</sub>, eg. 0.02% Tween 20<sup>TM</sup>

15 6. Reconditioning solution: 0.1 M NaOH, eg. 0.02% Tween 20<sup>TM</sup>

7. Storage buffer Oligo (dT)<sub>25</sub>: 250 mM Tris-HCl, pH 8, 20 mM EDTA, 0.1% Tween-20, 15 mM NaN<sub>3</sub>

#### Example of the reagents used for a mRNA purification

20 1. Binding buffer: 20 mM Tris-HCl, pH 7.5, 1.0 M LiCl, 2 mM EDTA, 15 mM NaN<sub>3</sub>, eg. 0.02% Tween 20<sup>TM</sup>

2. Washing buffer: 10 mM Tris-HCl, pH 8.0, 0.15 mM LiCl, 1 mM EDTA, 15 mM NaN<sub>3</sub>, eg. 0.02% Tween 20<sup>TM</sup>

5. Elution solution: 2 mM EDTA, pH 8.0, 15 mM NaN<sub>3</sub>, eg. 0.02% Tween 20<sup>TM</sup>

#### Example of reagents used for RNA isolation

25 1. 4 M guanidium isothiocyanate, 25 mM sodium citrate pH 7.0, 0.5% N-lauryl sarcosine, 0.01 M β-mercaptoethanol

**Example of reagents used for a DNA direct purification**

1. Particle suspension in Lysis buffer (eg. 50 mM Tris-HCl, pH 7.2, 50 mM EDTA, 3% SDS, 1% 2-mercaptoethanol; 50 mM KCl, 10 - 20 mM Tris-HCl, 2.5 mM MgCl<sub>2</sub>, pH 8.3, 0.5 Tween 20<sup>TM</sup>, 100 µg/ml Proteinase K; 100 mM Tris-HCl, pH 8.5, 5 mM EDTA, 1% SDS, 500 µg/ml Proteinase K) containing 15 mM NaN<sub>3</sub>
2. Washing buffer containing 15 mM NaN<sub>3</sub> and eg. 0.02% Tween 20<sup>TM</sup>
3. Resuspension buffer containing 15 mM NaN<sub>3</sub> and eg. 0.02% Tween 20<sup>TM</sup>

**Example of the purification process of PCR products by a magnetic particle processor at room temperature**

- 10 The reagents are dispensed into a subsequent wells of a plate.

**Example of a reagent configuration:**

- Well 1. Sample (biotinylated DNA, PCR amplicons)  
 Well 2. Streptavidin coated magnetic particles in washing buffer  
 Wells 3 - 5. Washing buffer
- 15 Well 6. NaOH  
 Well 7. TE buffer  
 Well 8. Distilled water

**Example of processing steps:**

- Well 2. Mixing, washing and collecting of particles, moving of them into well 3
- 20 Well 3. Washing of particles, moving of them into well 4  
 Well 4. Washing of particles, moving of them into well 1  
 Well 1. Sample incubation 10', moving of particles into well 4  
 Well 4. Washing of particles, moving of them into well 5  
 Well 5. Washing of particles, moving of them into well 6
- 25 Well 6. Incubation 5' in melting solution, moving of particles into well 4  
 Well 4. Washing of particles, moving of them into well 5  
 Well 5. Washing of particles, moving of them into well 7  
 Well 7. Rinsing of particles, moving of them into well 8  
 Well 8. Releasing of particles

### The effect of surface tension releasing agent (STRA) in collecting and releasing steps of magnetizable particles

Streptavidin coated magnetic particles (sizes: Scigen streptavidin 3  $\mu\text{m}$ ; Scigen; SPHERO<sup>TM</sup> streptavidin 4 - 4.5 $\mu\text{m}$ , Spherotec, Dynabeads M-280 streptavidin 2.8  $\mu\text{m}$ , Dynal) were saturated with biotinylated alkaline phosphatase (Calbiochem, San Diego, CA) for 1 h at +37 °C. Saturated particles were first washed to remove the unbound alkaline phosphatase and were then used to examine the effect of STRA in collecting and releasing steps of a magnetic particle processor. The instrument settings of these examples were adjusted from 20  $\mu\text{l}$  to 200  $\mu\text{l}$  and the capacity range of the processor was 1 - 24 samples per run. The processor utilized a rod magnet (cylindrical NdFeB axially magnetized, length 2 mm, width 3 mm) in polypropene tube (outer width 4.5 mm).

Briefly, the particles were processed by releasing and collecting them from well to well so that the whole process comprised of 10 steps. The amount of particles, which remained into the wells after the collection, was estimated with alkaline phosphatase assay. Samples (10  $\mu\text{l}$ ) from each well were transferred to an empty microtitration plate (round-bottomed wells, width 6.5 mm).

In this assay alkaline phosphatase saturated particles (0.016  $\mu\text{g}$  - 1  $\mu\text{g}$  particles / 10  $\mu\text{l}$  diluent) were used as standards. Into the wells containing 10  $\mu\text{l}$  samples and standards were added 100  $\mu\text{l}$  pNPP-substrate diluted in diethanolamine (DEA) buffer (Labsystems). The substrate was incubated for 15 minutes at +37 °C with continuous shaking (900 rpm) in Labsystems iEMS Incubator/Shaker. The reaction was stopped by adding 100  $\mu\text{l}$  1M NaOH into each well and the absorbances at 405 nm were measured by photometer (Labsystems Multiskan).

The amount of remaining particles was determined from a linear standard curve and finally results were expressed as percentage of initial amount of particles (0.2 mg/well).

In Fig 1. is shown the effect of detergent (Tween 20<sup>TM</sup>) in different concentrations. The degree of remaining particles (Scigen streptavidin) were over 3% / well, when surface tension releasing agent was not added into the collecting and releasing buffer. When the detergent concentration was  $\geq 0.00125\%$ , the particles were collected efficiently.



In Fig 2. is shown the effect of salt and saccharose in collecting and releasing buffer. By adding these components into the buffer, the collection of particles (Scigen streptavidin) was more efficient.

5 In Fig 3. is shown the effect of a protein (casein) which was improving the collecting steps of particles (SPHERO<sup>TM</sup> streptavidin) in some degree.

In Fig. 4 is shown the effect of detergent (Tween 20 <sup>TM</sup>) when the magnetic particles of different suppliers were used.

## Claims

1. A process for the purification of a substance, wherein
  - material containing the substance, and magnetic particles coated or treated with a reagent which binds the particles to the substance are dispensed in a first medium,
  - 5 - a binding reaction is let to take place, in which reaction the substance is bound to the particles, and
  - a magnetic probe is pushed into the medium, whereby the particles adhere to the probe, and the probe together with the particles and the substance bound to them is transferred to a second medium, and if desired, separated from the second medium
  - 10 and transferred to a third medium, characterized in that
  - a surface tension releasing agent is dispensed at least on of the mediums, preferably at least to the first medium, and most preferably to all mediums, before the probe and the particles are transferred from it.
- 15 2. A method according to claim 1, wherein the surface releasing compound is a tenside, alcohol, protein, or a salt or carbohydrate.
3. A method according to claim 1 or 2, wherein the surface tension releasing compound is a tenside, such as a detergent.
4. A method according to claim 3, wherein the concentration of the tenside is
  - 20 0.001 - 0.5% (w/v), preferably 0.005 - 0.1% (w/v), and most preferably 0.01 - 0.05% (w/v).
5. A method according to claim 1 or 2, wherein the surface tension releasing compound is a protein.
6. A method according to claim 5, wherein the concentration of the protein is
  - 25 0.1 - 10% (w/v), preferably 0.25 - 5% (w/v), and most preferably 0.5 - 2% (w/v).
7. A method according to claim 1 or 2, wherein the surface tension releasing compound is a salt.
8. A method according to claim 7, wherein the concentration of the salt is 0.1 - 10 M, preferably 0.1 - 7 M.
- 30 9. A method according to any of claims 1-8 for the purification of cells, viruses, subcellular organelles, proteins, or nucleic acid materials.

10. A method according to claim 9 for the purification of nucleic acid materials.
11. A method according to any of claims 1-10, wherein the size of the magnetic particles is less than 50  $\mu\text{m}$ , preferably 0.1 - 10  $\mu\text{m}$ , and most preferably 1 - 5  $\mu\text{m}$ .
12. A method according to any of claims 1-11, wherein the concentration of the magnetic particles is 0.01 - 5 mg/ml, preferably 0.05 - 3 mg/ml, and most preferably 0.2 - 2 mg/ml.
13. A method for separating magnetic particles by means of a magnetic probe from a medium, characterized in that a surface tension releasing agent is dispensed into the medium before the particles are separated.
14. A method for improving the adherence of magnetic particles from a liquid medium to a magnetic probe to be pushed into the medium, characterized in that a surface tension releasing agent is dispensed into the medium before the particles are adhered to the probe.

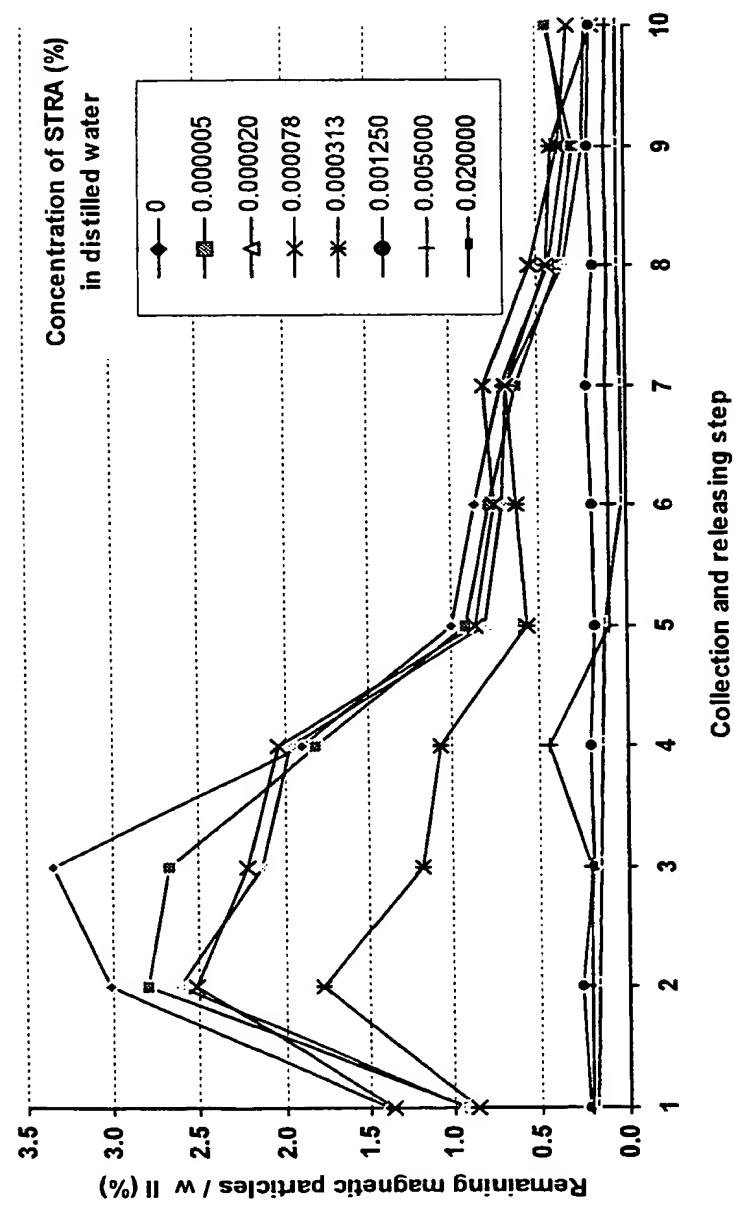


Fig. 1

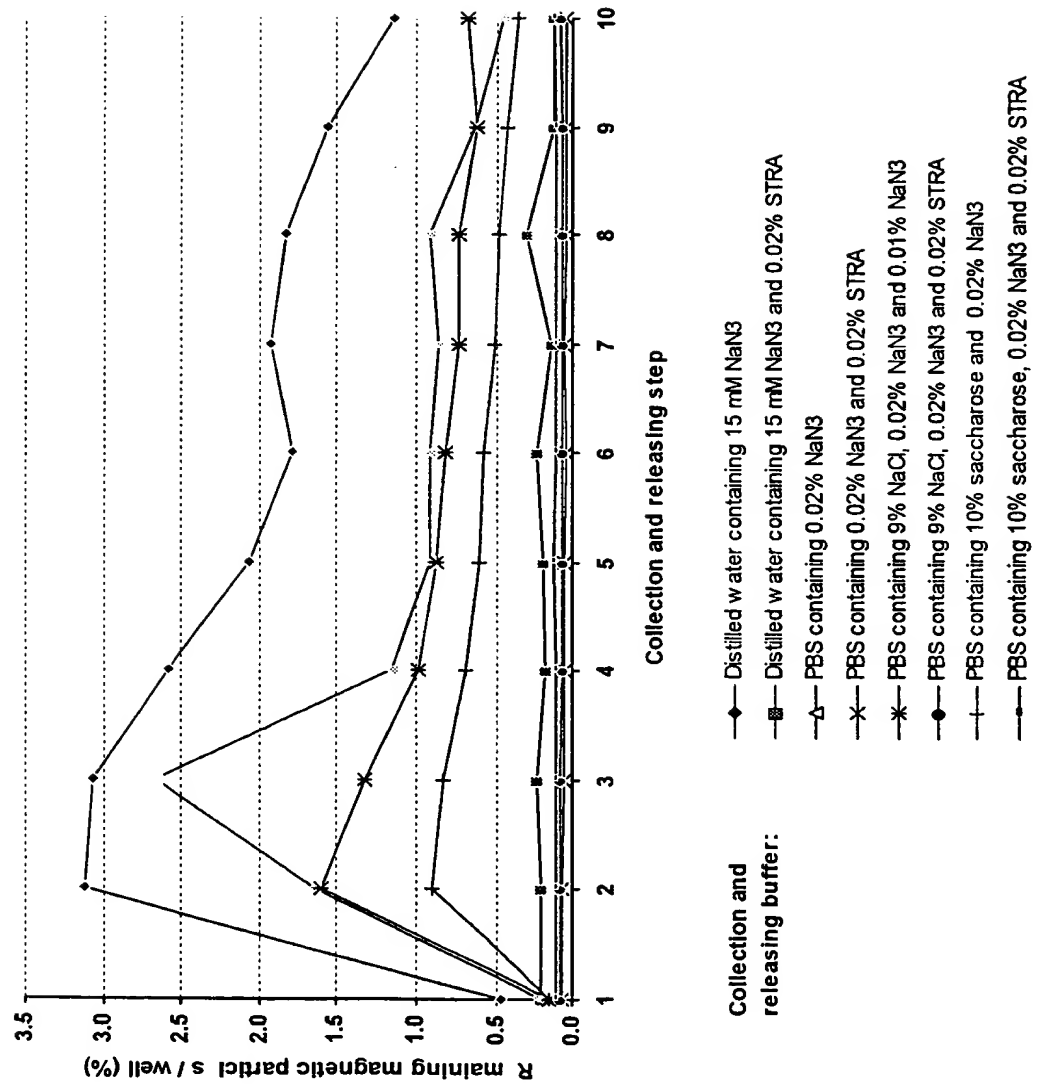


Fig. 2

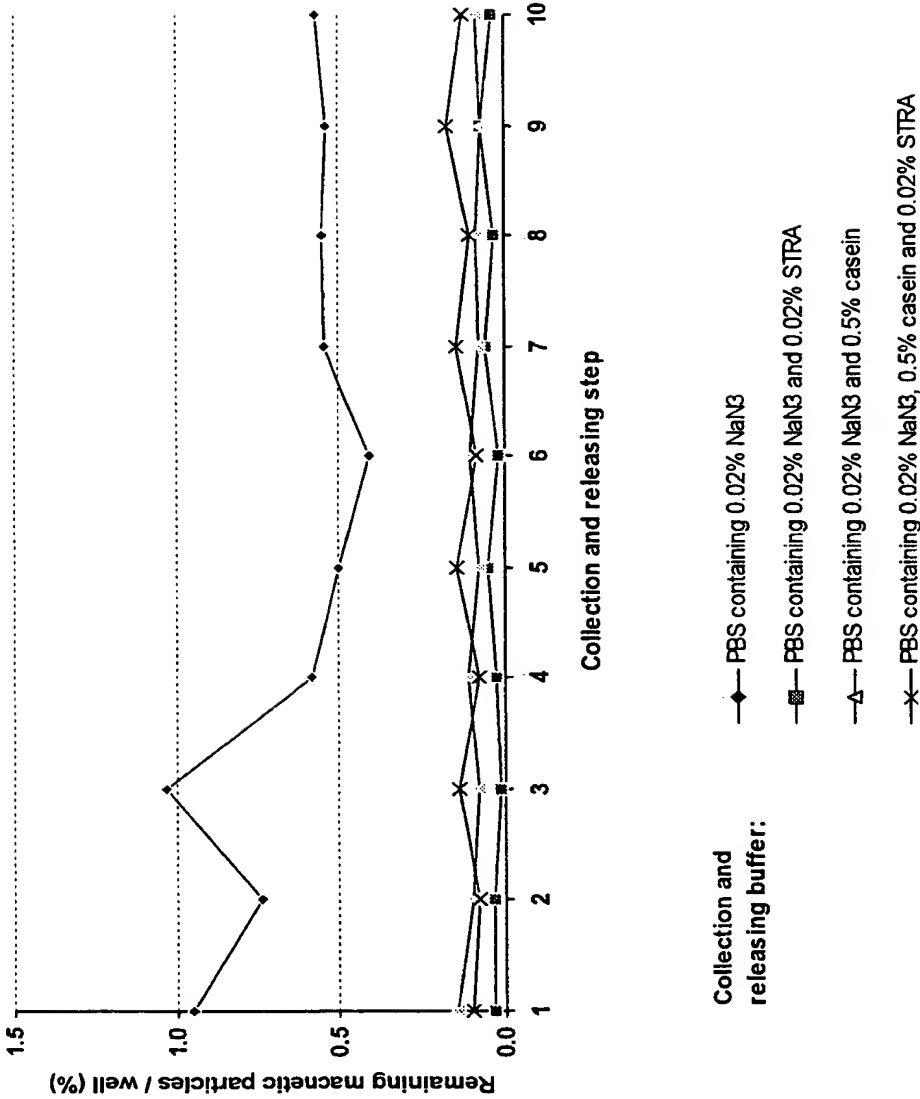


Fig. 3

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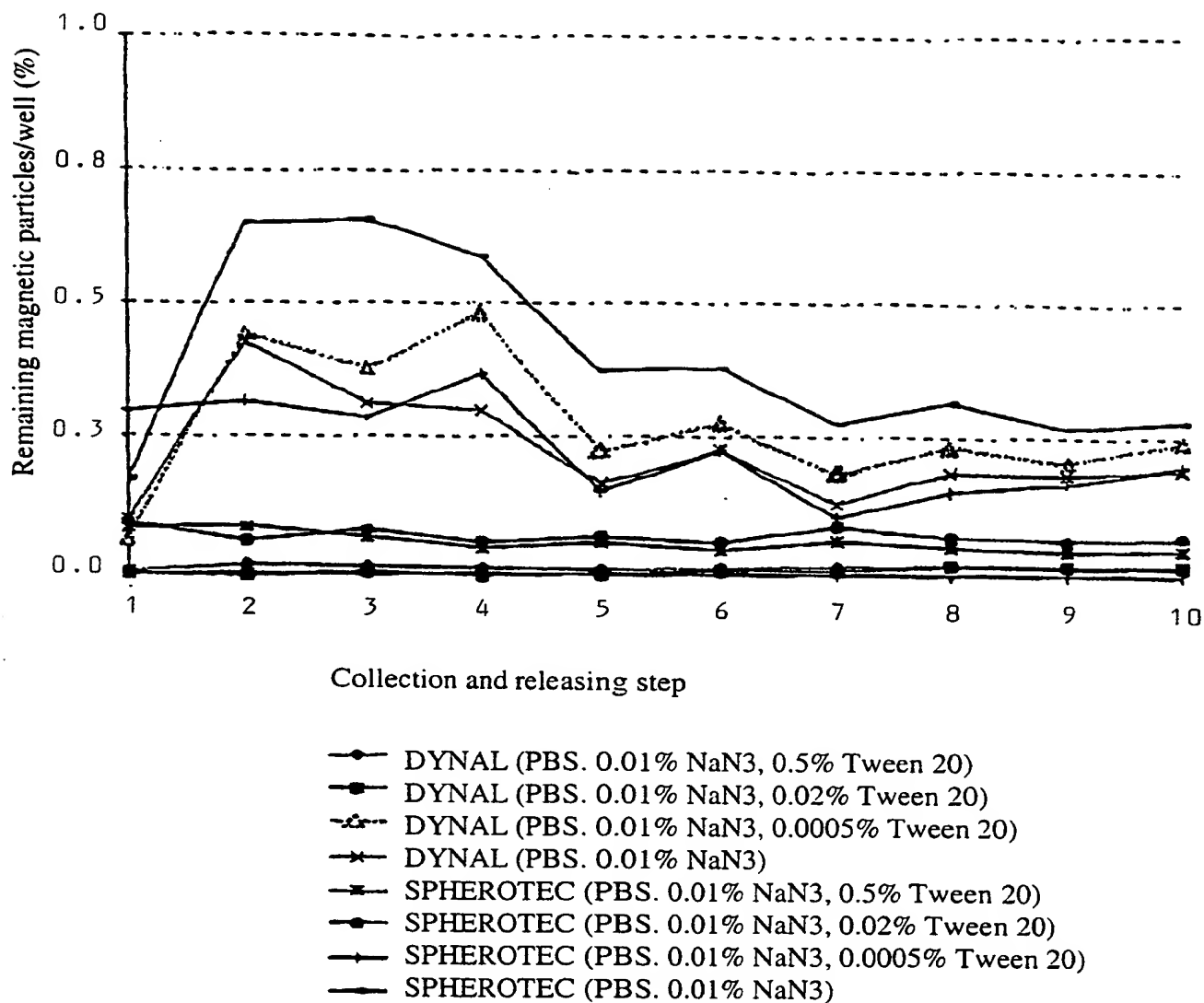


Fig. 4

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 00/00031

## A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/543

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9618731 A2 (DYNAL A/S), 20 June 1996 (20.06.96), see abstract; page 6, line 23 - page 12, line 9  --	1-14
X	US 5705628 A (TREVOR HAWKINS), 6 January 1998 (06.01.98), see abstract; column 1, line 24 - column 2, line 60; column 5, line 29 - column 6, line 27  --	1-14
A	WO 9831840 A1 (PROMEGA CORPORATION), 23 July 1998 (23.07.98), see abstract; page 7, lines 9-21; page 8, lines 2-7; page 8, line 30 - page 9, line 12; page 12, line 1 - page 13, line 8; page 17, line 20 - page 21, line 12  --	1-14

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document but published on or after the international filing date	"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

Date of mailing of the international search report

10 May 2000

17 -05- 2000

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 00/00031

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Microbiology, Volume 140, 1994, Anil Wipat et al, "Monoclonal antibodies for Streptomyces lividans and their use for immunomagnetic capture of spores from soil", page 2067 - page 2076, see abstract  --	1-14
A	WO 9612959 A1 (LABSYSTEMS OY), 2 May 1996 (02.05.96), see abstract  -- -----	1-14

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/FI00/00031**

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

**see next sheet**

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/FI00/00031**

According to PCT Rule 13.1 and 13.2, the international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). In order to achieve unity of invention the single general inventive concept must involve a "special technical feature" i.e. a technical feature that is common to the inventions and defines a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

With regard to the documents cited in the International Search Report, the present application is considered to consist, *à posteriori*, of the following inventions:

1. A process for the purification of a substance characterised in that magnetic particles coated with a reagent are used; a binding reaction takes place; a magnetic probe is pushed into the medium in order to transfer the particles from one solution to another; a surface tension releasing agent is dispersed in the medium before the probe and the particles is transferred from it. In the first invention the surface releasing compound is a tenside. This invention is disclosed in claims 1-4 and 9-14 (partially).
2. A process for the purification of a substance characterised in that magnetic particles coated with a reagent are used; a binding reaction takes place; a magnetic probe is pushed into the medium in order to transfer the particles from one solution to another; a surface tension releasing agent is dispersed in the medium before the probe and the particles is transferred from it. In the first invention the surface releasing compound is a protein. This invention is disclosed in claims 1-2, 5-6 and 9-14 (partially).
3. A process for the purification of a substance characterised in that magnetic particles coated with a reagent are used; a binding reaction takes place; a magnetic probe is pushed into the medium in order to transfer the particles from one solution to another; a surface tension releasing agent is dispersed in the medium before the probe and the particles is transferred from it. In the first invention the surface releasing compound is a salt. This invention is disclosed in claims 1-2, 7-8 and 9-14 (partially).

It is considered that all the inventions could be searched within one fee.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

02/12/99

International application No.  
**PCT/FI 00/00031**

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9618731 A2	20/06/96	AU 706211 B	10/06/99
		AU 4182996 A	03/07/96
		CA 2207608 A	20/06/96
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		JP 10508099 T	04/08/98
		NO 971805 A	18/04/97
		US 5942124 A	24/08/99
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